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Studies on the Chemistry of Lichens

II.* Umbilicin, an Arabitol Galactoside from *Umbilicaria pustulata* (L.) Hoffm.

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In connection with investigations on lichen acids the low-molecular carbohydrate constituents of some lichens have been studied. From *Umbilicaria pustulata* (L.) Hoffm. D-mannitol and another substance of m.p. 138—139° and showing $[\alpha]_D^{20}$ −81° (in water) were obtained. For the latter substance, which does not seem to be described in the literature, we propose the name umbilicin.

Umbilicin was easily soluble in water and had a low solubility in ethanol and moist acetone. It crystallized very slowly from supersaturated solutions; to obtain complete separation when the substance was not pure the solutions had to be kept several months, although one or two days were sufficient in the case of pure samples. Umbilicin did not reduce Fehlings solution. On the paper chromatogram it gave a single spot which lay just above that of mannitol and could be developed with lead tetraacetate [1]. After acid hydrolysis of the substance two spots were obtained, one of which could be developed with the usual sugar reagents (ammoniacal silver nitrate or aniline hydrogen phthalate) and was identical with the spot given by D-galactose on the same chromatograms. The second spot could not be developed with aniline hydrogen phthalate but did react with lead tetraacetate. After periodate oxidation on the paper, this spot could be developed with iodide-starch [1] but not with Schiff's reagent. This behaviour is typical of polyols, which give only formaldehyde and formic acid on periodate oxidation; the formaldehyde volatilises during

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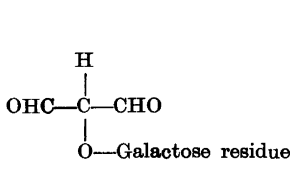
the oxidation and thus no colour is produced with Schiff's reagent. The position of the second spot, much below that of mannitol, indicated that it was due to a pentitol.

The two components of the hydrolysate were separated on a hydrocellulose column and isolated in a crystalline state. The fast-moving polyol was identified as D-arabitol. Its melting point, 98—100°, was not depressed on admixture with an authentic sample of D-arabitol, and the specific rotation in acidified ammonium molybdate solution was + 131°, agreeing with the value of —130° reported by Richtmyer and Hudson [2] for L-arabitol. The slow-moving sugar was identified as D-galactose by its specific rotation at equilibrium, + 76°, and by oxidation to mucic acid.

The hydrolysis experiments thus showed that umbilicin is a galactoside of D-arabitol, and analysis values for the substance and its acetate were consistent with the formula of an arabitol monogalactoside.

On periodate oxidation of umbilicin, 10 moles of oxidant were consumed, while one mole of galactose and one mole of arabitol should together consume only 9 moles. The high value observed can only be explained by the reaction proceeding through an intermediate containing an activated hydrogen atom; the latter is oxidized to an hydroxyl group, after which the whole molecule is degraded to one-carbon units. This abnormal type of periodate oxidation was first observed by Ahlborg [3] with maltose, and has been further studied by Huebner, Ames and Bublitz [4], who demonstrated that it occurs when a suitably activated hydrogen atom is present, *e.g.* in malonic acid. If the arabitol galactoside is a pyranoside, which is a very reasonable assumption, the third carbon atom of the arabitol must be glycosidically linked to the galactose in order to give an intermediate with an active hydrogen atom (I).



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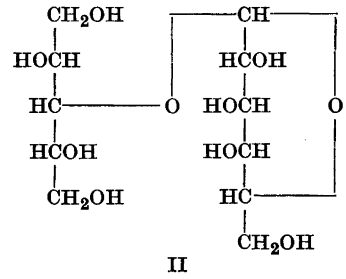

II

Further, the optical rotations of umbilicin and of its octaacetate ($[\alpha]_D^{20}$ —20° in chloroform) make it very probable that the substance is a β-galactoside. Even though it is impossible to calculate the contribution of the D-arabitol part of the molecule, it is very improbable that it would have a negative

value, high enough to more than equalise the high positive value of an α-galactoside. Thus the most probable structure of umbilicin would appear to be 3-D-arabitol β-D-galactopyranoside (II).

Umbilicaria pustulata is rather rich in carbohydrate constituents. In addition to D-mannitol and umbilicin it contains a polysaccharide, pustulin [5], composed of glucose units. Umbilicin seems to be the first glycoside isolated from a lichen and is of a type not frequently found in nature. A more careful investigation of the carbohydrate constituents of lichens is therefore justified from both biochemical and taxonomical points of view.

EXPERIMENTAL

Isolation of mannitol and umbilicin. Air-dried, ground *Umbilicaria pustulata* (500 g) (collected in the archipelago of Blekinge, Sweden) was continuously extracted with ether for about two days in order to remove the lichen acids. The extraction was then continued with acetone. After three hours the dark-brown acetone solution was discarded and the extraction continued with fresh acetone for 5 days. The extract deposited colourless crystals, which were separated and dissolved in water. Coloured impurities were removed by passing the aqueous solution through a small column of aluminium oxide. The acetone solution was concentrated to dryness, the residue shaken with water and the aqueous solution decolorized as before. The combined aqueous solutions were concentrated to dryness and the residue subjected to fractional crystallization from ethanol. By this procedure mannitol (1.5 g) was obtained in a pure state, m.p. 161—162° alone or on admixture with an authentic specimen, but it proved impossible to obtain the other component in a pure state by recrystallization alone. When most of the mannitol had been extracted, however, it occasionally happened that the umbilicin crystallized as hemispherical aggregates of stout prisms, which could be mechanically separated from the mannitol crystals and purified by further recrystallization. By tedious manipulation, 0.61 g of umbilicin, m.p. 136—137°, was finally obtained. (The total amount of umbilicin was certainly higher, the optical rotation of the combined aqueous solutions indicating a content of about 2.5 g.) Further recrystallizations yielded the pure substance, m.p. 138—139°, $[\alpha]_D^{20}$ −81° (water, c = 2).

$C_{11}H_{22}O_{10}$ (314)	Calc.	C	42.1	H	7.02
	Found	»	41.2	»	7.05

On periodate oxidation 1 mole of the substance consumed 10.2 moles of oxidant.

Umbilicin octaacetate. A small amount of umbilicin was acetylated with acetic anhydride and pyridine. The acetate was recrystallized from methanol, giving an almost quantitative yield of the octaacetate, m.p. 84—85°, $[\alpha]_D^{20}$ −20° (chloroform, c = 2).

$C_{11}H_{14}O_{10}$ $(OCCH_3)_8$ (650.6) Calc. CH_3CO 52.9 Found CH_3CO 53.0

Hydrolysis of umbilicin. Umbilicin (250 mg) was hydrolyzed with 0.2 *N* sulfuric acid (5 ml), at 100° for 16 hours, the solution freed from acid with barium carbonate and concentrated to a sirup which was then dissolved in butanol-ethanol, 1 : 1 (7 ml). This solution was introduced on to the top of a hydrocellulose column (8 cm^2 × 28 cm) saturated with butanol-ethanol-water (40 : 2 : 9), and eluted with the same solvent. The eluate was divided into fractions of 7 ml, which were examined for carbohydrates with the

reagents mentioned above. Fractions 47—72 contained the polyol, fractions 73 and 74 traces of both components and 75—150 the reducing sugar, although in fractions 110—150 only very small amounts were present.

Fractions 47—72 were combined, concentrated and crystallized from acetone-ethanol (1 : 1). D-arabitol (78 mg) of m.p. 98—100°, undepressed on admixture with an authentic specimen, was obtained. The specific rotation determined in acidified ammonium molybdate solution according to the method of Richtmyer and Hudson [2] was + 131° (c = 0.2).

Fractions 74—150 were combined, concentrated and crystallized from ethanol. After one recrystallization fairly pure D-galactose (47 mg), showing m.p. 148—150° and the final rotation $[\alpha]_D^{20}$ + 76° (water, c = 0.2), was obtained. The slight impurities in the galactose were probably due to the fact that the umbilicin used for the hydrolysis was not perfectly pure and that the impurities accumulated preferentially in the sugar fraction. The galactose was characterized by oxidation to mucic acid in the ordinary way.

SUMMARY

From the lichen *Umbilicaria pustulata* (L.) Hoffm. D-mannitol and a galactoside, umbilicin, have been isolated. Umbilicin has been shown to be 3-D-arabitol β-D-galactopyranoside, on the assumption that furanoside structures may be excluded.

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